In the Claims:

Please add new claims 36 and 37.

36. The molecular circuit of any of claims ~~1-6~~, 9-11, and 22-23, wherein the activity of the transcription factor is regulated by a second stimulus other than stress.

~~37~~. 36 The molecular circuit of any of claims 15-17, wherein the activity of the first or the second transcription factor is regulated by a second stimulus other than stress.

REMARKS

Claim Amendments

Addition of new claims 36 and 37 was requested in the previous Amendment filed September 15, 2000 (Paper No.11), but necessary fees were inadvertantly not paid. The request is reiterated respectfully, and payment of fees is enclosed. As explained in Paper No.11, support for the two claims is found on p.21, lines 29-30, on p.22, entire page, and in Fig.4.

Rejection of Claim 1 Under 35 U.S.C. 102(b)

The rejection of claim 1 under 35 U.S.C. 102(b) as being anticipated by Moonen Chrit (WO98/06864) was maintained. The Examiner explained that "the reference discloses a therapeutic gene under the control of a heat shock promoter which is interpreted to be one of the nucleic acid molecules, the endogenously present heat shock promoter and heat shock transcription factor are being interpreted as being the other nucleic acid molecule".

Applicant respectfully disagrees with this argument. The composition of claim 1 comprises

"(a) a first nucleic acid molecule that comprises a gene encoding a transcription factor and a first promoter activatable by stress and by the transcription factor, wherein the first

promoter and the transcription factor gene are operably linked, and (b) a second nucleic acid molecule that comprises a gene of interest and a second promoter..."

According to claim 1, the transcription factor gene and the first promoter activatable by stress and by the transcription factor need to be operably linked, i.e., the expression of the transcription factor gene needs to be under the control of the first promoter. See also p.10, lines 25-26 of the specification. Endogenous heat shock factors are expressed from constitutively active genes. The question of whether heat shock factor genes are controlled by a heat shock promoter has been specifically examined by several research groups including Applicant's. Rabindran, S.K. et al. 1991. Cloning and expression of a human heat shock factor, HSF1. Proc.Natl.Acad.Sci. USA 88: 6906-6910. Sarge, K.D. et al. 1991. Cloning and characterization of two mouse heat shock factors with distinct inducible and constitutive DNA-binding ability. Genes Dev. 5: 1902-1911. Articles are enclosed as Exhibits A and B. The unambiguous answer obtained by all was that expression of heat shock factor genes is not heat-regulated in several organisms. Hence, endogenous heat shock factor genes are not operably linked to heat shock promoters. Therefore, an endogenous heat shock factor gene cannot substitute for one of the elements of the composition of claim 1. Withdrawal of the rejection is respectfully requested.

Rejection of Claim 9 Under 35 U.S.C. 112, Second Paragraph

Claim 9 was rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner explained that it was "not clear what transcription factor activates the third promoter: is it the first or the second transcription factor?" Applicant believes that the Examiner may have misread claim 9. Claim 9 relates to a circuit comprising three elements, a gene of interest controlled by a transcription factor and two copies of the gene for the transcription factor, of which two copies one is regulated by a heat shock gene promoter and the other by a promoter responsive to the transcription factor. Thus, the circuit gives rise to the synthesis of a single type of

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transcription factor from two separate gene copies. Withdrawal of the rejection is respectfully requested.

Rejections of Claims 1-33 and 35 Under 35 U.S.C. 112, First Paragraph

The Examiner made two related enablement rejections under 35 U.S.C. 112, first paragraph. The Examiner stated that the "ordinary artisan would not recognize that applicant was in possession of all possible circuit combinations at the time the invention was made", and that the specification "does not reasonably provide enablement for all promoters and transcription factors. The specification does not enable any person skilled in the art to which it pertains, or to which it is most nearly connected, to practice the invention commensurate in scope with these claims." The Examiner explained her rejections as follows: "The claims are drawn to a molecular circuit that serves as a cascade of activation to eventually express the gene of interest. The specification prophetically mentions the potential universe of combinations that could be made yet does not disclose enough information in the form of working examples to guide the ordinary artisan to make a working combination that would be commensurate with the scope of the claims. Specific information regarding the promoters and transcription factors is required", and "One cannot extrapolate the teachings of the specification to the scope of the claims because the claims are broadly drawn to any molecular circuit that uses a stress inducible promoter which in turn activates a transcription factor, that activates a promoter that activates a gene of interest or that activates another transcription factor which activates a promoter and a gene of interest. In order to make such an activation cascade, detailed knowledge of the promoters and their activation sites and the transcription factor binding sites are needed. In addition it is essential that one knows how much of the promoter sequence is necessary to fulfill the requisite function..."

Applicant greatly appreciates that the Examiner granted him a brief telephone interview on March 22, 2001. The interview focused on the above rejections under 35 U.S.C. 112, first paragraph. The conclusions were that a declaration presenting experimental data relating to circuits of the invention would be helpful and that arguments and explanations

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should be provided highlighting how the circuits of the declaration were prepared using information from the specification.

Before presenting Applicant's arguments countering the above rejections, it may be useful to comment on the Examiner's characterization of the compositions of the invention. The Examiner wrote that the invention encompasses "any molecular circuit that uses a stress inducible promoter which in turn activates a transcription factor, that activates a promoter that activates a gene of interest or that activates another transcription factor which activates a promoter and a gene of interest." This characterization ignores an essential element of the circuits of the invention. The circuits of the invention are "devices" to achieve sustained activation of a gene of interest by a single, transient stress. As is well known, a gene of interest controlled by a heat shock gene promoter can only be transiently activated by a transient stress. Even long term stress exposure does not result in sustained activation, because at high levels of stress cells are killed and at low levels feedback regulation kicks in and heat shock gene promoters are inactivated. See also p.2, lines 22-25, p.20, lines 9-12, p.24, lines 23-30, etc. In the circuits of the invention this rapid inactivation of a gene of interest subsequent to a transient stress is avoided by the introduction of a transcription factor that controls the expression of the gene of interest. The transcription factor is initially expressed under the control of a heat shock gene promoter and is subsequently, i.e., subsequent to the activating stress, maintained by a feedforward loop (transactivation of the transcription factor gene by the transcription factor itself). This feedforward loop is a component of all circuits of the invention, and the presence of this element limits drastically the universe of circuits claimed.

I am now turning to the above-mentioned rejections under 35 U.S.C. 112, first paragraph. In the field of biochemistry the names of proteins are functional names. For example, a protein called glutamate dehydrogenase is an enzyme that converts glutamate to alpha-keto-glutarate and ammonia. A protein can only be given this name if an experiment has shown that the protein in fact possesses this enzymatic activity. Similarly, a transcription factor is a protein that has been shown experimentally to bind to elements within a promoter and to enhance transcription from the promoter. Hence, it is understood by the

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ordinary artisan that, if a protein is called a transcription factor, there must exist previous knowledge about at least one promoter that is the target of the factor. In the absence of this knowledge, the protein could not have been experimentally shown to be a transcription factor and hence could not be properly called a transcription factor. The specification additionally provided a specific definition of "transcription factor" that corresponds to this common understanding of the term. This definition is found on p.8, lines 28-30: "As used herein, a "transcription factor" is a protein that binds to a nucleic acid and thereby influences its transcription by altering rates of transcription initiation or elongation." This definition implies that a protein must have been shown to have the mentioned properties before it can be called "transcription factor". This showing could not have been made without the knowledge of at least one promoter that is responsive to the factor. Thus, available to the artisan in connection with each transcription factor of the invention is at least one specific nucleic acid that is a target and is specifically transactivated by the transcription factor.

In order to make nucleic acid molecules of the invention, nothing more is required than the joining of an available promoter segment known to be transactivated by the selected transcription factor and/or by stress to a segment containing the gene for the transcription factor or a gene of interest using routine subcloning procedures. Thirty something years after subcloning technology has come into general use, every beginning graduate student in biochemistry, molecular biology or a related field is capable of carrying out such routine manipulations. It is noted that the person skilled in the art is considered to be a notional team of postdoctoral workers who have a far greater level of expertise.

Thus, the preparation of the circuits of the invention involves assembly of known elements using routine subcloning procedures. It follows that all that is needed to enable the invention is sufficient guidance on which combinations of these elements to make to obtain molecular circuits that have the property of achieving sustained activation of a gene of interest by a transient stress. Applicant's argument is that the specification provides ample guidance, enabling the invention over the entire claimed scope. In support of this argument, Applicant is filing concurrently a declaration under 37 C.F.R. 1.132 that

reports experiments with five different circuits of the invention involving four different transcription factors. Applicant's declaration shows that, because as was discussed before the name "transcription factor" is a functional name requiring prior demonstration of transactivation of a responsive promoter, Applicant was able for each transcription factor to obtain sufficient information from the scientific or commercial literature to identify a promoter responsive to the transcription factor. Applicant further shows that he was able to build each circuit by combining known promoters, transcription factor genes and genes of interest using nothing but the most routine subcloning procedures and following the instructions provided in the specification for making the correct combinations. Finally, Applicant demonstrates that each circuit so assembled had the desired property of achieving sustained activation of the gene of interest by a transient stress.

While the Examiner is respectfully invited to carefully examine the accompanying declaration, the significance of the data of the declaration is explained hereinafter. The specification distinguished three types of circuits, the first incorporating a heat shock gene promoter-regulated mutated heat shock factor, the second a heat shock gene promoter-regulated chimeric transcription factor derived from a heat shock factor and the third a heat shock gene promoter-regulated transcription factor not related to a heat shock factor. The declaration includes examples of all three types of circuits.

The first circuit presented in the declaration under "Type 1 Circuit" is the same circuit as that described in the specification on p.13, lines 11-25. It consists of a construct containing a cDNA gene for HSF1d202-316 (referred to herein and in the declaration as HSF1(+)) linked to a (human) *hsp70B* promoter and a construct containing a luciferase gene linked to the same promoter, exactly as described in the specification. HSF1d202-316 had been characterized by Zuo et al. (Mol.Cell.Biol. 14: 7557 (1994); Mol.Cell.Biol. 15: 4319 (1995)), and the *hsp70B* promoter by Voellmy et al. (Proc.Natl.Acad.Sci.USA 82: 4949 (1985)) and Schiller et al. (J.Mol.Biol. 203: 97 (1988)).

The constructs of the circuit were prepared by routine methods closely similar to those described under Example 1 (p.32-34). These manipulations were completely

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straightforward. In the first step of the preparation of construct hsp70-HSF1(+), a BglII-HindIII fragment known to contain a complete hsp70B promoter as well as RNA leader sequences (Voellmy et al., 1985; Schiller et al., 1988) was inserted into common vector pcDNA3.1 (Invitrogen Corp.), replacing a segment of the vector containing a CMV promoter. In the second step, a HindIII-EcoR1 fragment known to contain the HSF1(+) sequence (Zuo et al. 1994; 1995) was introduced into the pcDNA3.1 polycloning site now located downstream of the *hsp70B* sequences. The construction of the gene of interest, hsp70-Luc, was even more simple and consisted of introducing a similar hsp70B promoter and RNA leader segment into the polylinker site upstream from a luciferase gene in a commercially available construct.

The circuit was analyzed and was found to possess the property of achieving sustained activation of the luciferase gene subsequent to a transient heat stress as explained in the specification on p.12, lines 11-20 and p.34, lines 16-24.

The second circuit also presented in the declaration under the heading "Type 1 Circuit" is the same as that described in Example 2 of the specification. The circuit consisted of the same hsp70B-HSF1(+) construct as that of the previous circuit and of hsp70-human growth hormone construct p17hGHdhfr specifically mentioned in the specification. p17hGHdhfr was described by Dreano et al. (Gene 49: 1 (1986)). The circuit was found to have the property of achieving sustained activation of the growth hormone gene subsequent to a transient heat stress as explained in the specification on p.35, lines 10-17.

The third circuit presented in the declaration under "Type 2 Circuit" is the same circuit as that described in the specification on p.19, lines 13 to p.20, line 8 and in Fig.2, except that two copies of the transcription factor gene were included, of which one was regulated by a heat shock gene promoter and the other by a LexA-responsive promoter. This use of two copies of the transcription factor gene in type 2 circuits was described in the specification on p.21, lines 5-11. For experiments aimed at demonstrating the proper operation of circuits of the invention (which was a major goal of the experiments reported in the declaration), the use of two separate transcription factor genes is highly desirable

because this allows for a comparison of complete circuits and circuits lacking a transcription factor-inducible transcription factor gene and, hence, for an estimation of the contribution of the feedforward loop central to the circuits of the invention. The type 2 circuit of the declaration consisted of a gene encoding chimeric transcription factor LexA-HSF1d202-316 (LexA-HSF1(+)) linked to a *hsp70B* promoter, a second copy of the same transcription factor gene linked to a LexA-responsive promoter and a luciferase gene (gene of interest) linked to the same LexA-responsive promoter. Chimeric transcription factor LexA-HSF1(+) as well as a promoter responsive to the factor (LexA-responsive promoter) were previously characterized (Zuo et al., 1994; 1995).

Preparation of the constructs of the circuit involved nothing but routine methods of subcloning. Preparation of the gene of interest construct consisted of inserting a fragment known to contain a LexA-responsive promoter and RNA leader sequences (Zuo et al., 1994; 1995) into the polylinker region of a commercial construct containing a luciferase gene. The construction of hsp70-LexA-HSF1(+) and of lexA-LexA-HSF1(+), respectively, involved the replacement in the commonly used vector pcDNA3.1 of a CMV promoter segment with of a known fragment containing complete *hsp70B* promoter sequences as well as RNA leader sequences (Voellmy et al., 1985; Schiller et al., 1988) and a known fragment containing a functional LexA-responsive promoter and RNA leader sequences (Zuo et al., 1994; 1995), respectively, followed by insertion in the polylinker region of the vector of a known fragment encoding transcription factor LexA-HSF1(+) (Zuo et al., 1994; 1995).

The circuit was analyzed in transfected cultured cells and was found to possess the property of achieving sustained activation of the luciferase gene subsequent to a transient heat stress essentially as explained in the specification on p.20, lines 3-8. Comparison of the activity of a complete circuit and an incomplete circuit lacking construct lexA-LexA-HSF1(+) revealed the critical importance of autoactivated expression of the chimeric transcription factor, i.e., the importance of the feedforward loop.

The fourth circuit presented in the declaration under "Type 3 Circuit" makes use of chimeric transcription factor GLVP. This factor that is unrelated to heat shock factors contains a GAL4 DNA-binding domain, a modified ligand-binding domain from a progesterone receptor and VP16 activation domains. The factor is activated by binding of mifepristone (RU486). Transcription factor GLVP as well as a synthetic promoter responsive to GLVP were described in Wang et al. Proc.Natl.Acad.Sci. USA 91: 8180 (1994), Wang et al. Gene Therapy 4: 432 (1997a) and Wang et al. Nature Biotechnology 15: 239 (1997b). The GLVP-responsive promoter contains a segment including 4 repeated GAL4 recognition sites that is joined to a basal promoter (referred to below as "17x4"). The fourth circuit contained a gene of interest (luciferase) linked to the 17x4 promoter (17x4-Luc) and 2 copies of the GLVP gene, one of which was linked to the *hsp70B* promoter (hsp70-GLVP) and the other to the 17x4 promoter (17x4-GLVP). Instructions for assembling circuits of this type are found on p.21, lines 16-28, of the specification. Examples are given on p.21, lines 16-28, on p.22, lines 4-30 and in Figs. 3 and 4. The inclusion of regulated transcription factors (such as GLVP in the present circuit) is mentioned in the specification on p.21, last two lines and p.22, first three lines, and is exemplified on p.22, lines 4-30 and in Fig.4.

Constructs for the fourth circuit were prepared using nothing but routine subcloning procedures. To construct 17x4-GLVP, a restriction fragment containing the GLVP gene obtained from construct pCEP4-GLVP (Wang et al., 1994) was first inserted into the polylinker site of commercial vector pcDNA3.1, and the GLVP sequences and adjacent BGH polyA sequences (from the vector) were subsequently PCR-amplified using primers designed based on the known sequence of pcDNA3.1 (available from the Invitrogen web site: www.invitrogen.com), ligated to a fragment containing the 17x4 promoter (Wang et al., 1994) and the ligation product inserted into the polylinker site of commercial vector SP72 (Promega). Construct hsp70-GLVP was made by sequential insertion into the polylinker site of SP72 of the entire *hsp70B* promoter present in construct OR173 (Voellmy et al., 1985) and the above GLVP-containing PCR fragment. To prepare 17x4-Luc, the 17x4 promoter from construct p17x4 TATA CAT (Wang et al., 1994, and a

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restriction map furnished by the authors) was inserted into the cloning site of commercial luciferase expression vector GL2B (Promega Corp.).

The circuit was analyzed in transfected cultured cells and was found to possess the property of achieving sustained activation of the luciferase gene subsequent to a transient heat stress in the presence of ligand mifepristone essentially as explained for the example of a type 3 circuit described on p.22, lines 4-30, of the specification. Comparison of the activity of a complete circuit and an incomplete circuit lacking construct 17x4-GLVP revealed the critical importance of autoactivated expression of the transcription factor, i.e., the importance of the feedforward loop. Ligand withdrawal resulted in an inactivation of the circuit as was explained on p.22, lines 23-24, of the specification.

The fifth circuit also presented in the declaration under "Type 3 Circuit" makes use of chimeric transcription factor VgEcR/RXR. This factor is unrelated to heat shock factors. It is a heterodimer of a modified insect ecdysone receptor and retinoid X receptor. The transcription factor and a promoter responsive to the transcription factor (E/GRE promoter) were described in literature distributed by Invitrogen Corp. The same information can also be obtained from the company's web site (www.invitrogen.com) under the heading "Ecdysone Inducible Expression System". Constructs containing transcription factor and vector sequences were purchased from Invitrogen Corp. The fifth circuit is essentially the same as that described on p.22, lines 4-30, of the specification and in Fig.4, except that the RXR gene was left under the control of a constitutively active promoter and the gene of interest was a luciferase gene instead of a cytokine gene. The circuit contained a construct containing a gene of interest controlled by the E/GRE promoter (E/GRE-Luc), a construct containing transcription factor subunit gene VgEcR functionally linked to the *hsp70B* promoter and a constitutively active RXR gene (hsp70-VgEcR-RXR), and a construct containing the VgEcR gene under the control of the E/GRE promoter (E/GRE-VgEcR).

Constructs for this fifth circuit were prepared using nothing but routine subcloning procedures. Hsp70-VgEcR-RXR was constructed by sequential insertion into the

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polylinker site of commercial vector SP72 of the entire *hsp70B* promoter present in construct OR173 (Voellmy et al., 1985) and of a PCR fragment containing the VgEcR gene, polyA sequences, an RSV promoter, the RXR gene and flanking polyA sequences amplified using primers designed based on the known sequence of Invitrogen's plasmid VgRXR and pVgRXR as a template. E/GRE-VgEcR was prepared by inserting a PCR-amplified VgEcR gene fragment (from pVgRXR) into the cloning site of E/GRE-containing Invitrogen plasmid IND. Construction of E/GRE-Luc involved subcloning of a luciferase gene fragment from commercial plasmid GL3B (Promega) into pIND.

The circuit was analyzed in transfected cultured cells and was found to possess the property of achieving sustained activation of the luciferase gene subsequent to a transient heat stress in the presence of ligand ponasterone as explained for the example of a type 3 circuit described on p.22, lines 4-30, of the specification. Comparison of the activity of a complete circuit and an incomplete circuit lacking construct E/GRE-VgEcR revealed the importance of autoactivated expression of the transcription factor, i.e., the importance of the feedforward loop. It is noted that the incomplete circuit displayed some "leakiness", i.e. some non-negligible activity. It is expected that this leakiness could be suppressed in a circuit assembled exactly as proposed on p.22 of the specification and in Fig.4 in which the RXR gene is also subjected to regulation by stress and the transcription factor, respectively. Ligand withdrawal resulted in an inactivation of the circuit as was explained on p.22, lines 23-24, of the specification.

The above-discussed examples that are described in more detail in the accompanying declaration amply demonstrate that, because as was discussed before the name "transcription factor" is a functional name requiring prior demonstration of transactivation of a responsive promoter, Applicant was able for each of four different transcription factors to obtain sufficient information from the scientific or commercial literature to identify a promoter responsive to the transcription factor. The discussion further showed that Applicant was able to build each of the five circuits by combining known promoters, transcription factor genes and genes of interest using nothing but the most routine subcloning procedures and following the instructions provided in the

specification for making the correct combinations. Finally, each circuit so assembled had the desired property of achieving sustained activation of the gene of interest by a transient stress. Thus, these multiple and diverse examples of working circuits of the invention demonstrate that the specification broadly enabled the circuits of the invention. Withdrawal of the rejections under 35 U.S.C. 112, first paragraph, is respectfully requested.

Applicant believes that the application is now in condition for allowance. If the Examiner believes that a telephone conversation will aid his review, the Examiner is kindly invited to call Applicant (who is his own attorney) at (305) 243-5815.

Respectfully Submitted,

Richard Voellmy

Reg. # 40,859